IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	:	10/517,686 Confirmation No. 3094
Applicants	:	Evert J. BUNSCHOTEN et al.
Filed	:	June 30, 2005
Title	:	METHOD OF TREATING OR PREVENTING IMMUNE MEDIATED DISORDERS AND PHARMACEUTICAL FORMULATION FOR USE THEREIN
Group Art Unit	:	1616
Examiner	:	Mei Ping CHUI
Customer No.	:	28289
Application No.	:	10/521,040 Confirmation No. 6630
Applicants	:	Herman J. T. COELINGH BENNINK et al.
Filed	:	August 16, 2005
Title	:	PHARMACEUTICAL COMPOSITION COMPRISING ESTETROL DERIVATIVES FOR USE IN CANCER THERAPY
Group Art Unit	:	1616
Examiner	:	Mei Ping CHUI
Customer No.	:	28289

DECLARATION

I, Herman J. T. COELINGH BENNINK, Ph.D., declare and state the following:

1. I am a co-inventor for the inventions claimed and recited in the above-captioned patent applications. A detailed listing of my publications, together with details of my education, are provided in my *curriculum vitae* which is attached as Exhibit A.

2. Based on my academic training and professional experience, I consider myself an expert in the field of estrogen-related therapies and treatments, and I was such a person in 2001 and 2002.

3. I am a co-author of articles relating to estetrol. One of these articles was published in CLIMACTERIC (May 2008) 11(Supp 1): 47-58, a copy of which is annexed hereto as Exhibit B. This article show that:

- Following single dose oral administration (0.1, 1, 10 and 100 mg) to early postmenopausal women, estetrol exhibited a dose-independent terminal elimination half-life of about 28 hours;
- In a competitive ligand binding assay there was no detectable binding of estetrol to human sex hormone binding globulin (SHBG); and
- Fluorometric assays in wild-type human HepG2 and Hep89 cells showed that estetrol does not stimulate ERα-mediated increases in SHBG production by these cells.

4. To the best of my knowledge, prior to June 11, 2002, there were no publicly available data about the terminal elimination half-life of estetrol, about estetrol's binding to SHBG or about estetrol's effect on SHBG production.

5. Before June 11, 2002 several scientific articles were published that describe the results of animal studies and *in vitro* studies in which pharmacological properties of estetrol were investigated. From these data one of ordinary skill would have concluded that estetrol has an estrogenic potency that is considerably lower than that of the natural estrogens estradiol and estriol. Exhibit C, for instance, reports that the relative values of association constants of estradiol and estetrol for estrogen receptors in human endometrial cytosol were 100:1.5. Exhibit D reports that in nonpregnant ewes, following intra-arterial (uterine) injections estetrol is 15 to 30 times less potent than estriol as an uterine vasodilator. Exhibit E reports that in rats estetrol administered by s.c. injection at 50 μg/100g body mass produced uterotropic effects that were smaller than those observed for estradiol or estriol injected at a 50 times lower dose.

6. The human studies and *in vitro* studies reported in Exhibit B have shown that estetrol is very different from the natural estrogens estradiol and estriol in that it has a much longer terminal elimination half-life in humans, little affinity for human SHBG and no stimulatory effect on SHBG-production in humans. These conclusions find support in the data presented in Exhibits F to H. Exhibit F shows that estradiol has a terminal elimination half-life of

about 30 minutes and Exhibit G shows that estriol has a terminal elimination half-life of 5-10 minutes. Exhibit H shows that estradiol is strongly bound by SHBG and that both estradiol and estriol stimulate SHBG production.

7. Estetrol's long terminal elimination half-life in humans, its low affinity for human SHBG and its non-stimulatory effect on human SHBG production contribute greatly to its pharmacological usefulness. Had this not been the case, estetrol's very low estrogenic potency would have pre-empted successful pharmaceutical applications of estetrol in humans. In other words, the unexpected discovery that estetrol is very different from other natural estrogens in that it has a very long terminal elimination half-life, shows little SHBG binding and does not stimulate SHBG-production provided the clue to its pharmaceutical usefulness in humans. Before June 11, 2002, however, one of ordinary skill in the art could not have been aware of these clues.

8. I declare further that all statements made herein are true to my knowledge; and that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Herman J. T. COELINGH BENNINK, Ph.D.

August 20, 2009
Date

Exhibits

A *Curriculum Vitae* of Herman J. T. Coelingh Bennink, Ph.D

B Coelingh Bennink et al., *Estetrol Review: profile and potential clinical applications*, Climacteric (2008); 11 (Suppl 1): 47-58

C Tseng et al., *Heterogeneity of saturable estradiol binding sites in nuclei of human endometrium. Estetrol studies.* J. Steroid Biochem. (1978); 9, 1145-118.

D Levine et al., *Uterine vascular effects of estetrol in nonpregnant ewes.* Am. J. Obstet. Gynecol. (1984); 148:73, 735-738.

E Holinka et al., *Comparison of effects of estetrol and tamoxifen with those of estriol and estradiol on the immature rat uterus.* Biol Reprod (1980) 22, 913-926.

F Leon Speroff, Robert H. Glass and Nathan G. Kase. *Clinical Gynecologic Endocrinology and Infertility.* Baltimore, Maryland, USA. Lippincott Williams & Wilkins (1999), 270

G White et al., *The pharmacokinetics of intravenous estradiol: A preliminary study,* Pharmacotherapy (1998), vol. 18, no. 6, 1343-1346

H Hammond et al. *Estetrol does not bind sex hormone binding globulin or increase its production by human HepG2 cells*, Climacteric (2008); 11 (Suppl 1): 1-6